PATENT COOPERATION TREATY

PCT

REC'D 20 MAR 2006
WIPO PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC116000	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/CA2004/001986	International filing date *(day/month/year)* 18 November 2004 (18-11-2004)	Priority date *(day/month/year)* 20 November 2003 (20-11-2003)

International Patent Classification (IPC) or national classification and IPC
IPC: ***C07D 413/06*** (2006.01), ***A61K 31/4412*** (2006.01), ***A61K 31/5377*** (2006.01), ***A61K 31/496*** (2006.01), ***A61P 39/00*** (2006.01), ***C07D 401/06*** (2006.01), ***C07D 213/81*** (2006.01)

Applicant
APOTEX INC. ET AL

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [x] *(sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      [x] sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. 1 and the Supplemental Box.
   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) ,containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No.I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [x] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [x] Box No. VIII Certain observations on the international application

Date of submission of the demand 16 June 2005 (16-06-2005)	Date of completion of this report 17 March 2006 (17-03-2006)
Name and mailing address of the IPEA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Authorized officer Marc De Vleeschauwer (819) 956-6127

BEST AVAILABLE COPY

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/001986

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[x] the international application in the language in which it was filed

[ ] a translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (Rules 12.3(a) and 23.1(b))

[ ] publication of the international application (Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

[ ] the international application as originally filed/furnished

[X] the description:

[X] pages 1-35, 37-39, 43-62 as originally filed/furnished

[X] pages* 36, 40-42 received by this Authority on 20 September 2005 (20-09-2005)

[ ] pages* received by this Authority on

[X] the claims:

[ ] pages as originally filed/furnished

[ ] pages* as amended (together with any statement) under Article 19

[X] pages* 63-66 received by this Authority on 20 September 2005 (20-09-2005)

[ ] pages* received by this Authority on

[X] the drawings:

[X] pages 1-6 as originally filed/furnished

[ ] pages* received by this Authority on

[ ] pages* received by this Authority on

[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

BEST AVAILABLE COPY

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/001986

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-17	YES
	Claims	none	NO
Inventive step (IS)	Claims	1-17	YES
	Claims	none	NO
Industrial applicability (IA)	Claims	1-17	YES
	Claims	none	NO

2. Citations and explanations (Rule 70.7)

D1 US 6,472,532
D2 US 6,472,229
D3 CA 2,287,907
D4 US 5,688,815

NOVELTY
D1 and D2 disclose processes for manufacturing 3-hydroxy-4-oxo-1,4-dihydropyridine-2-carboxamides.
D3 discloses 3-hydroxy-4-oxo-1,4-dihydropyridine-2-carboxamides having iron chelating proprieties, and oral pharmaceutical formulations comprising such to treat diseases of excess of iron.
D4 discloses 3-hydroxy-4-oxo-1,4-dihydropyridine substituted with a heteroaryl-carbonyl at position 2, and oral pharmaceutical formulations comprising such to treat diseases of excess of iron.
None of D1-D4 discloses cycloalkyl substituent either on the dihydropyridine nitrogen atom or on the nitrogen atom of the carboxamide susbtituent in position 2. Therefore, claims 1 to 17 present novelty over D1-D4 and comply with Article 33(2) PCT.

INVENTIVE STEP
D3 is the closest prior art document. The difference between the present application and that document is the presence of at least one cycloalkyl substituent on the nitrogen atom of the dihydropyridine ring or on the nitrogen atom of the carboxamide susbtituent. The closest substituent on the corresponding atoms in D3 is *aliphatic hydrocarbon group* which is exemplified as being straight or branched alkyl. D3 does not teach toward the presence of cycloalkyl substituent on the nitrogen atoms. The same applies for the compounds disclosed in D1, D2 and D4. Therefore, claims 1 to 17 present an inventive step and do comply with Article 33(3) PCT

INDUSTRIAL APPLICABILITY
The subject matter of claims 1 to 17 define new compounds that could be used to treat diseases of excess of iron, formulations and process thereof. Therfore, it is considered to be industrially applicable and is thus fulfilling the requirements of Article 33(4) PCT.

BEST AVAILABLE COPY

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Page 14, line 20, of the description does not comply with Rule 5.1(a)(ii) because of the presence of a web address. The nature of the Internet makes information it contains volatile and changing. Therefore, a reference to a web page does not constitute a valid reference for the description of the background art of the invention.

The paragraph on lines 20 to 24 of page 51 of the description does not comply with Article 6 PCT, because it implies that the protection sought goes beyond the scope of the claims.

BEST AVAILABLE COPY

EXAMPLE 10: pKa determination for Apo6619 by potentiometric titration

The pKa values of ligands were determined by potentiometric titration when a ligand concentration greater than $1x10^{-2}$ M in water could be prepared. In a typical experiment, the sample solution ($2.67x10^{-2}$ M) was prepared by the following method: Apo6619 (92.6 mg) was weighed into a 25-ml beaker, followed by the addition of 0.1 M NaCl (15 ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Nitrogen gas was then allowed to bubble through the solution. 1.000 N Hydrochloric acid (624 µl, 1.5 equivalent) was added to the solution to give pH 1.88. The solution was allowed to equilibrate at 22°C for 60 minutes.

The solution was then titrated against 1.000 N NaOH at 22°C to reach pH 11.8. For each addition of base, the solution was allowed to equilibrate until a constant pH reading was reached. The volume of the base added and the pH reading were recorded for each measurement. 137 measurements were taken to finish the experiment.

The data set of pH vs. base volume was analyzed using Hyperquad 2000 (Version 2.1, Peter Gans, University of Leeds). Given the model: $L^- + H^+ \leftrightarrow LH$ (pKa_1) and $LH + H^+ \leftrightarrow LH_2^+$ (pKa_2), the pKa values of Apo6619 were optimized as $pKa_1 = 8.6$ and $pKa_2 = 2.5$.

EXAMPLE 11: pKa determination for Apo6617 by spectrophotometric titration

The pKa values of ligands can be determined by spectrophotometric titration when both the conjugated acid and base absorb in the UV-Visible region. In a typical experiment, the sample solution was prepared by the following method: Apo6617 (0.792 mg) was weighed into an 80-ml beaker, followed by the addition of 0.1 M NaCl (50 ml). The mixture was sonicated for 5 minutes to give a clear colorless solution. Nitrogen gas was allowed to bubble through the solution. 1.000 N NaOH (50 µl) was added to give pH 10.9. The solution was allowed to equilibrate at 22°C for 1 hour. A sipper

BEST AVAILABLE COPY

AMENDED SHEET

acid solutions were purchased from VWR Scientific Products. MOPS (3-[N-Morpholino]propanesulfonic acid) was purchased from Sigma-Aldrich.

B. Determination of stepwise formation constants for Fe-Apo6619 system by spectrophotometric titration. Apo6619 is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide.

Stepwise formation constants for M^{n+}-ligand systems were determined by spectrophotometric titration when metal complexes have a strong absorbance in the visible region due to ligand to metal charge transfer. In a typical experiment, the sample solution was prepared according to the following method: Apo6619 (10.7 mg) was weighed into an 80-ml beaker, followed by the addition of 0.1 M NaCl (50 ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Iron stock solution (atomic absorption standard, Aldrich, 496 µl, 8.93E-06 moles) was pipetted into the solution followed by the addition of 1.000 N NaOH (137 µl). The molar ratio between the total iron and the total Apo6619 was 1:5.4. The mixture was allowed to equilibrate at room temperature overnight. Nitrogen was allowed to bubble through the solution. 1.000 N Hydrochloric acid (3 ml) was then added to the solution to give pH 1.5. The solution was allowed to equilibrate at 22°C for 3 hours.

A sipper system was used for the circulation of the sample solution between the beaker and the flow cell.

The sample solution was titrated against standard NaOH solutions at 22°C to reach pH 6.89. After each addition of base the solution was allowed to equilibrate until a constant pH reading was reached. The pH and the UV-Vis spectrum were recorded for each measurement. For each measurement enough base was added so that there was a slight increase in the absorbance of the spectrum. The solution was titrated until there was no obvious increase in the spectra after several subsequent additions of base. Altogether 64 measurements were taken to finish the experiment.

The resulting data set was then analyzed using pHAB. Given the model: $L^- + H^+ \leftrightarrow LH$ (pKa_1), $LH + H^+ \leftrightarrow LH_2^+$ (pKa_2), $Fe^{3+} + L^- \leftrightarrow FeL^{2+}$ (K_1), $FeL^{2+} + L^- \leftrightarrow FeL_2^+$ (K_2), $FeL_2^+ + L^- \leftrightarrow FeL_3$ (K_3), and $\beta_3 = K_1K_2K_3$, the

BEST AVAILABLE COPY

stepwise formation constants for Fe-Apo6619 system were optimized as log K_1 = 12.5(1); log K_2 = 11.6(1); log K_3 = 9.5(1); log β_3 = 33.6(2).

C. Determination of stepwise formation constants for Al-Apo6619 system by potentiometric titration.

Stepwise formation constants for M^{n+}-ligand system were determined by potentiometric titration when metal complexes (≥ 0.002 M) do not precipitate during titration. In a typical experiment, the sample solution was prepared by the following method: Apo6619 (31.91 mg) was weighed into a 25-ml beaker followed by the addition of 0.1 M NaCl (18.9 ml). The mixture was sonicated for 10 minutes to give a clear colorless solution. Aluminum stock solution (atomic absorption standard, Aldrich, 971 µl, 3.59 x 10^{-5} mole) was pipetted into the solution followed by the addition of 1.000 N NaOH (229 µl) to give pH 8.56. The molar ratio between the total Aluminum and the total Apo6619 was 1:4. For M^{2+} metals, a molar ratio of 1:3 was used. Nitrogen was allowed to bubble through the solution. The mixture was allowed to equilibrate at 22°C for 2 hours. 1.000 N Hydrochloric acid (264 µl) was then added to the solution to give pH 2.20. The solution was allowed to equilibrate at 22°C for 1 hour.

The solution was titrated against 1.000 N NaOH at 22°C to reach pH 11.0. For each addition of base, the solution was allowed to equilibrate until a constant pH reading was reached. The volume of the base added and the pH reading were then recorded for each measurement. 93 measurements were used in the experiment.

The data set of pH vs. base volume was analyzed using Hyperquad 2000. Given the model: $L^- + H^+ \leftrightarrow LH$ (pKa_1), $LH + H^+ \leftrightarrow LH_2^+$ (pKa_2), $Al^{3+} + L^- \leftrightarrow AlL^{2+}$ (K_1), $AlL^{2+} + L^- \leftrightarrow AlL_2^+$ (K_2), $AlL_2^+ + L^- \leftrightarrow AlL_3$ (K_3), and $\beta_3 = K_1K_2K_3$, the stepwise formation constants for Al-Apo6619 system were optimized as log K_1 = 12.6(2); log K_2 = 9.2(1); log K_3 = 8.4(1); log β_3 = 30.2(2).

Calculation of pM^{n+}

pM^{n+} is defined as $-\log[M(H_2O)_m]^{n+}$ at physiological conditions, *i.e.*: pH 7.4, a ligand concentration of 10 µM, and a metal concentration of 1 µM.

BEST AVAILABLE COPY

To calculate pM^{n+} for a ML_n system, β_n and pKa values are needed (β_n are the formation constants for $M^{n+} + n\ L^- \leftrightarrow ML_n$; pKa are the equilibrium constants for $L^- + n\ H^+ \leftrightarrow LH_n^{(n-1)+}$). The pM^{n+} can be calculated by using Hyss software (Hyperquad Stimulation and Speciation software: HYSS2 © 2000 Protonic Sofware).

The data obtained from the above determinations for compounds of formula I can be found in Table 1 and 2.

EXAMPLE 15: Evaluation of compounds of formula I in iron overloaded rats

Effectiveness of Apo6619 and Apo6617 in Promoting Urinary and Fecal Iron Excretion in the Iron Overloaded Rat.

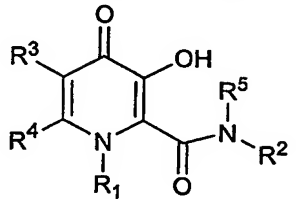
The purpose of this study was to determine the effectiveness of Apo6619 and Apo6617 in promoting iron excretion in the iron overloaded rat model. Iron overloading was achieved by administration of iron dextran. Iron overloading using iron dextran has previously been used to assess chelator efficacy in mice (Kontoghiorghes G. J., *Mol Pharmacol.* **1986,** *30(6)*, 670-3; Bartfay et al., *Cardiovasc Res.* **1999**, *43(4),* 892-900), gerbils (Hershko et al., *J. Lab Clin Med* **2002**, *139*, 50-58), rats (Rakba N. *Biochem Pharmacol.* **1998**, 55(11):1796-1806) and primates (Bergeron et. al., *Blood*, **1992**, *79(7)*,1882-1890). The iron loading regime used in this study results in a 20-fold increase in liver iron and a 3.8-fold increase in cardiac iron levels in male rats. Previous studies in this model have demonstrated that this model is not associated with significant abnormalities in animal weight gain, food consumption, clinical chemistry or hematology parameters.

Experimental Protocol:

Six male Sprague-Dawley rats (weighing between 200-250 gms) were received from Charles River Laboratories, Montreal, Quebec, Canada. Rats were iron loaded by administration of iron dextran intraperitoneally at a dose of 100 mg/kg, twice weekly for a period of 4 weeks for a total of 8 injections

CLAIMS:

1. A 3-hydroxypyridin-4-one compound of formula I:



I

wherein:

R^1 is X with the proviso that R^2 is Y;

or

R^1 is T with the proviso that R^2 is W;

or

R^1 is X with the proviso that R^2R^5N when taken together, form a heterocyclic ring selected from piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl, wherein the group piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl is either unsubstituted or substituted with one to three C_1 to C_6 alkyl groups;

X is C_3-C_6 cycloalkyl;

Y is selected from the group consisting of C_3-C_6 cycloalkyl, C_1 to C_6 alkyl and C_1 to C_6 alkyl monosubstituted with a C_3-C_6 cycloalkyl;

T is C_1 to C_6 alkyl;

W is C_3-C_6 cycloalkyl;

R^3 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl;

R^4 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl;

R^5 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl;

and/or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein R^1 is X with the proviso that R^2 is Y.

BEST AVAILABLE COPY

3. A compound of claim 2 wherein X is C_3-C_6 cycloalkyl, Y is C_1 to C_6 alkyl and R^5 is hydrogen or methyl.

4. A compound of claim 3 wherein X is cyclopropyl, Y is methyl, R^3 is hydrogen, R^4 is methyl and R^5 is hydrogen, said compound is 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide.

5. A pharmaceutical composition comprising 1-cyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid methylamide and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition of claim 5 in a form suitable for oral use.

7. A compound of claim 2 wherein X is C_3-C_6 cycloalkyl, Y is C_3-C_6 cycloalkyl and R^5 is hydrogen.

8. A compound of claim 7 wherein X is cyclopropyl, Y is cyclopropyl, R^3 is hydrogen, R^4 is methyl, said compound is *N*,1-dicyclopropyl-3-hydroxy-6-methyl-4-oxo-1,4-dihydropyridine-2-carboxamide.

9. A compound of claim 3 wherein X is cyclopropyl, Y is methyl, R^3 is hydrogen, R^4 is methyl and R^5 is methyl, said compound is 1-cyclopropyl-3-hydroxy-*N*,*N*,6-trimethyl-4-oxo-1,4-dihydropyridine-2-carboxamide.

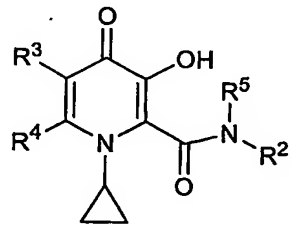
10. A compound according to claim 1 wherein R^1 is T with the proviso that R^2 is W.

11. A compound of claim 10 wherein T is methyl, W is cyclopropyl, R^3 is hydrogen, R^4 is methyl and R^5 is hydrogen, said compound is 3-

BEST AVAILABLE COPY

hydroxy-1,6-dimethyl-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid cyclopropylamide.

12. A 3-hydroxypyridin-4-one compound of formula IA:



IA

wherein:

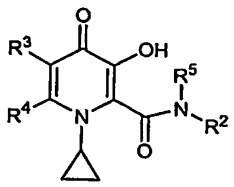
R^2 is selected from the group consisting of C_3-C_6 cycloalkyl, C_1 to C_6 alkyl and C_1 to C_6 alkyl monosubstituted with a C_3-C_6 cycloalkyl;

R^5 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl or R^5R^2N when taken together, form a heterocyclic ring selected from piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl, wherein the group piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl is either unsubstituted or substituted with one to three C_1 to C_6 alkyl groups;

R^3 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl; and

R^4 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl.

13. A process for the preparation of a compound of formula IA



IA

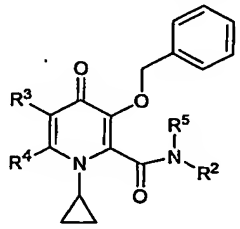
wherein:

R^2 is selected from the group consisting of C_3-C_6 cycloalkyl, C_1 to C_6 alkyl and C_1 to C_6 alkyl monosubstituted with a C_3-C_6 cycloalkyl;

R^5 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl or R^5R^2N when taken together, form a heterocyclic ring selected from piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl, wherein the group

BEST AVAILABLE COPY

piperidinyl, morpholinyl, pyrrolidinyl or piperazinyl is either unsubstituted or substituted with one to three C_1 to C_6 alkyl groups;
R^3 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl;
R^4 is selected from the group consisting of hydrogen and C_1 to C_6 alkyl;
which includes the step of deprotecting a benzyl group in a hydrogenation reaction of a compound of the general formula of 3-benzyloxypyridin-4-one, or its hydrochloride salt,



wherein R^2, R^5, R^5R^2N, R^3, R^4 are as defined in claim 1.

14. The process of claim 13 wherein the hydrogenation reaction is effected with palladium on charcoal or palladium hydroxide on charcoal and hydrogen in an inert solvent selected from the group consisting of methanol, ethanol and isopropanol.

15. A pharmaceutical composition comprising a compound according to claim 1 and a physiologically acceptable carrier.

16. A pharmaceutical composition according to claim 15 in a form suitable for oral use.

17. Use of a compound according to claim 1 in the manufacture of medicament in the treatment of a medical condition related to a toxic concentration of iron.

BEST AVAILABLE COPY